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Identification, Characterization, and Nucleotide Sequence of the F17-G Gene, Which Determines Receptor Binding of *Escherichia coli* F17 Fimbriae

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Enterotoxigenic *Escherichia coli* strains express fimbriae which mediate binding to intestinal mucosal cells. The F17 fimbriae mediate binding to *N*-acetylglucosamine-containing receptors present on calf intestinal mucosal cells. These fimbriae consist of F17-A subunit peptides. Analysis of the F17 gene cluster indicated that at least the F17-A, F17-C, F17-D, and F17-G genes are indispensable to obtain adhesive F17 fimbriae (unpublished data). Genetic evidence is presented that the F17-G protein, a minor fimbrial component, is required for the binding of the F17 fimbriae to the intestinal villi. The F17-G gene was cloned and sequenced. An open reading frame of 1,032 bp encoding a polypeptide of 344 amino acids, starting with a signal sequence of 22 residues, was localized. The F17-G mutant strain produced F17 fimbriae which were morphologically identical to the fimbriae purified from strains which contained the intact F17 gene cluster. However, this F17-G mutant could no longer adhere to calf villi. The F17-G locus was shown to act in *trans*: transformation of the F17-G mutant strain, still expressing the genes F17-A, F17-C, and F17-D, with a vector expressing the F17-G gene restored the binding activity of this mutant strain.

Enterotoxigenic *Escherichia coli* strains provoke diarrhea by colonizing the small intestine of the host and by secreting one or several enterotoxins (7). The colonization of the gut by enterotoxigenic *E. coli* occurs via one or more host-specific fimbrial adhesins (7, 11, 15, 30). Bovine fimbrial antigens which are actually incorporated in vaccines to protect newborn calves from diarrhea are the K99, F41, and F17 fimbriae (5). The F17 fimbriae were formerly called FY or Att25 and mediate binding to *N*-acetylglucosamine (NAG)-containing receptors present on intestinal mucosal cells (11, 20, 31, 35). It is interesting that the F17-positive pathogenic *E. coli* represent a significant part (20%) of the calf isolates in Belgium. The cloning of the F17 genome was recently described, and the sequence of the F17-A gene encoding the F17 pilus subunit was established (21). It was shown for type 1, P, and S fimbriae of *E. coli* and type 3 fimbriae of *Klebsiella pneumoniae* that the genetic determinant of adhesive function was distinct from the gene encoding the fimbrial subunit. Mutants and subclones were found that expressed fimbriae but were not adhesive or that lacked fimbriae but remained adhesive (8, 13, 16, 19, 22–24, 26, 27, 29, 32, 33, 39).

In this study, we demonstrated the dissociation between F17 fimbrial production and NAG-specific adhesion. The adhesion of F17 fimbriae was mediated by the F17-G protein. The F17-G gene was localized and sequenced. Insertional inactivation of the F17-G gene resulted in bacteria producing piliated cells which could no longer adhere to intestinal villi, while F17-A mutants lacked both properties. The F17-G mutants expressed fimbriae which were morphologically identical to the fimbriae present at the surface of strains containing the entire F17 gene cluster. Introduction of the F17-G gene into the F17-G mutant strain resulted in NAG-dependent adhesion of this strain to villi. This result indicates that one *trans*-acting product restored the binding properties of the nonadhering mutant.

MATERIALS AND METHODS

Bacterial strains, media, and plasmids. *E. coli* JM101 (12) and K514 (4) were used as recipient strains for transformation. *E. coli* HB101 was used in studies of the binding properties of the genetic constructions (2). *E. coli* ORN103 was used in minicell analysis (34). Bacteria were grown in LB medium at 37°C (17). Carbenicillin (100 μg/ml) and chloramphenicol (20 μg/ml) were the antibiotics used in selective media.

Plasmid constructs and their phenotypes are described in Table 1 and Fig. 1. The pUC8, pUC9 (41), and pACYC184 (3) plasmids were the cloning vectors.

DNA sequencing. The DNA samples were obtained by the method of Swinghamer (37). DNA was sequenced by the chemical method of Maxam and Gilbert (28) and the dideoxy-chain termination method of Sanger et al. (36).

Electron microscopy. The negative-staining drop method was used. Observations were made with a transmission electron microscope. Immunogold electron microscopy was done exactly as described previously (20), using either purified antibodies directed against F17 fimbriae or adsorbed antibodies directed against F17-G protein (G-specific antisera). This adsorption was done by incubating complete F17 antibodies with the piliated, F17-G-negative strain HB101 (pPLHD53) for 24 h at 4°C; this procedure was repeated at

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TABLE 1. Phenotypes of pPLHD2 and derivatives

Construction	Gene(s) present	ELISA	EM[a]	Adhesion to:	
				Calf villi	Eupergit-C
pPLHD2	F17-A, -D, -C, and -G	+	+	+	+
pPLHD52	F17-A, -D, -C, and -G	+	+	+	+
pPLHD50	F17-D, -C, and -G	−	−	−	−
pPLHD51	F17-D, -C, and -G	−	−	−	−
pPLHD53	F17-A, -D, and -C	+	+	−	−
pPLHD55	F17-A, -D, and -C	+	+	−	−
pPLHD101	F17-G	−	−	−	−
pPLHD101 + pPLHD55[b]	F17-A, -D, -C, and -G	+	+	+	+

[a] Electron microscopy.
[b] In *trans* complementation.

least three times. To control the elimination of the antibodies directed against the pilus structure (F17-A), we performed an enzyme-linked immunosorbent assay (ELISA) technique using microtiter plates coated with purified F17-A protein. When we used the G-specific antisera in ELISA with piliated bacteria mutated in the F17-G gene (pPLHD53), no binding of the antibodies could be demonstrated, indicating that the ELISA technique was below the detection limit of the F17-G proteins. Therefore, electron microscopy was used to visualize the binding of F17-G-specific antibodies.

In vitro adhesion. Intestinal villi were prepared as described by Girardeau (10). In addition, an alternative adhesion assay was done using Eupergit-C ovomucoid conjugate (40). Adhesion was monitored under a phase-contrast microscope (×600). Inhibition of adhesion was obtained with 50 mM NAG (final concentration). α-Methyl-D-mannoside was used as the control sugar at a final concentration of 100 mM.

Linker insertion mutagenesis. A phosphorylated *Xba*I linker with the -TAG- nonsense codon in all reading frames was used for linker insertion mutagenesis (25). The sequence of the linker was d(pCTAGTCTAGACTAG) (New England Biolabs Inc.). Plasmid DNA was linearized with the *Bcl*I or *Asp* 718 restriction enzyme followed by ligation of the linker DNA. The obtained transformants were digested with *Xba*I to verify insertion of the *Xba*I linker.

Analysis of protein expression. Both in vivo and in vitro labeling of proteins was done. Plasmids to be analyzed were transformed into the minicell-producing strain ORN103. Preparation of minicells and labeling were as described previously (1, 38).

In vitro labeling was done with plasmid DNA centrifuged twice on a $CsCl_2$ gradient in order to obtain RNA-free DNA. Plasmid DNA was treated in a bacterial cell-free coupled transcription-translation system (Amersham). Labeling was done with [^{35}S]methionine or [^{3}H]leucine. The gels were fixed, stained, destained (18), and exposed to X-ray film.

Nucleotide sequence accession number. The nucleotide sequence of the F17-G gene will appear in the GenBank

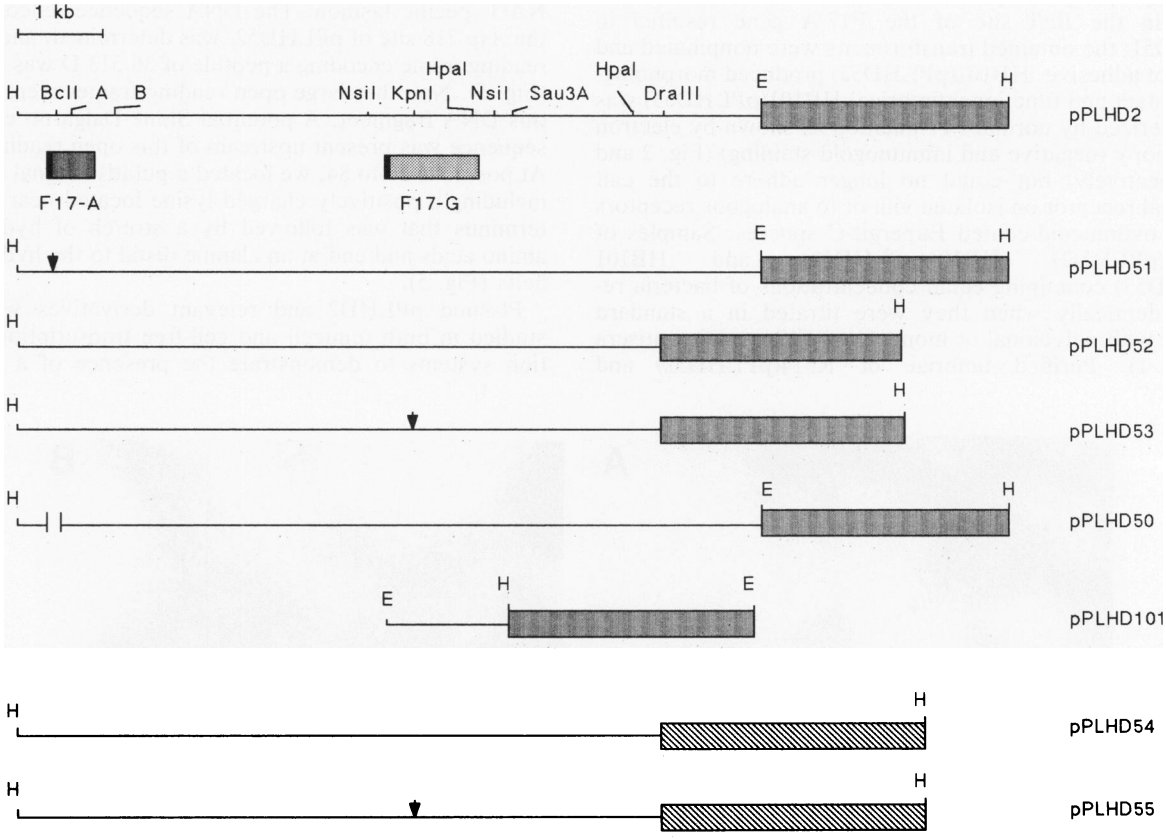


FIG. 1. Physical and genetic map of pPLHD2 and derivatives. Hatched bars show pACYC184 vector, gray bars are pUC8 vector, and filled bars represent the signal sequences of F17-A and F17-G. Arrows indicate *Xba*I linker insertion. Line interruption represents DNA deletion. Abbreviations: A, *Asu*II; B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII.

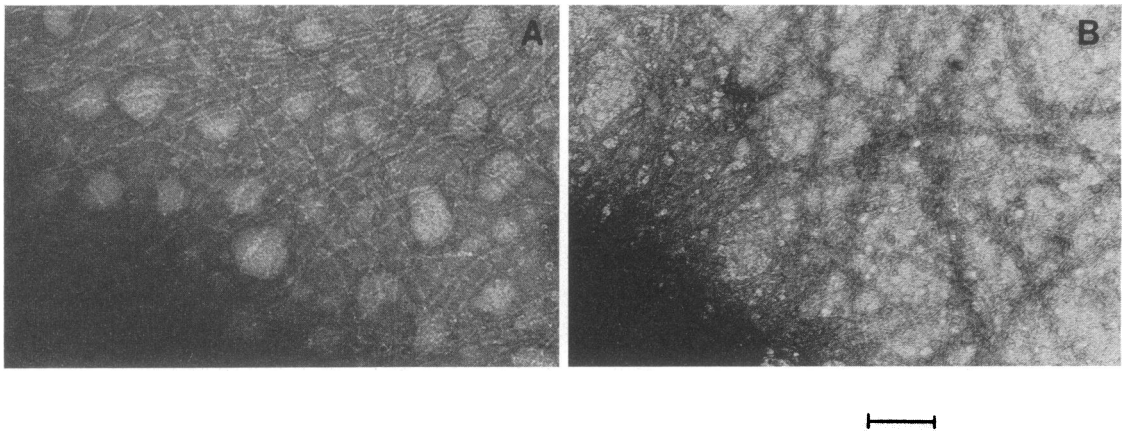


FIG. 2. Electron micrographs of *E. coli* HB101(pPLHD52) (A) and *E. coli*(pPLHD53) (B). Bar, 0.1 μm.

nucleotide sequence data bases under the accession number M62503.

RESULTS

Construction and analysis of deletions, subclones, and linker insertion mutants of pPLHD2. A physical map of pPLHD2 and derivatives is shown in Fig. 1. The phenotypes of pPLHD2 and derivatives are given in Table 1. All derivatives of pPLHD2 with the exception of HB101(pPLHD52) and HB101(pPLHD53) were characterized by loss of both binding property and pilus formation. Insertion of the *Xba*I linker in the *Bcl*I site of the F17-A gene resulted in pPLHD51; the obtained transformants were nonpiliated and were not adhesive. HB101(pPLHD52) produced morphologically intact and functional fimbriae. HB101(pPLHD53) was characterized by normal F17 piliation as shown by electron microscopy (negative and immunogold staining) (Fig. 2 and 3, respectively) but could no longer adhere to the calf intestinal receptor on isolated villi or to analogous receptors on the ovomucoid-coated Eupergit-C spheres. Samples of HB101(pPLHD2), HB101(pPLHD52), and HB101 (pPLHD53) containing equal concentrations of bacteria reacted identically when they were titrated in a standard ELISA with polyclonal or monoclonal F17-specific antisera (Table 1). Purified fimbriae of K514(pPLHD52) and K514(pPLHD53) migrated at the same position on sodium dodecyl sulfate (SDS)-polyacrylamide gels (data not shown). Immunogold electron microscopy studies with adsorbed F17 antibodies (the F17-G-specific fraction) demonstrated significantly more gold particles on the fimbriae encoded by pPLHD52 than on the fimbriae encoded by pPLHD53 (Fig. 4).

Identification and localization of F17 adhesin gene. An *E. coli* HB101 strain containing the *Xba*I linker at the unique *Asp* 718 site of pPLHD52 produced morphologically normal fimbriae which were unable to adhere to calf villi in a NAG-specific fashion. The DNA sequence, encompassing the *Asp* 718 site of pPLHD52, was determined, and an open reading frame encoding a peptide of 36,513 D was identified (Fig. 5). No other large open reading frames were found in this DNA fragment. A potential Shine-Dalgarno consensus sequence was present upstream of this open reading frame. At positions 18 to 84, we located a putative signal sequence including a positively charged lysine located near the NH_2 terminus that was followed by a stretch of hydrophobic amino acids and end at an alanine distal to the hydrophobic helix (Fig. 5).

Plasmid pPLHD2 and relevant derivatives were then studied in both minicell and cell-free transcription-translation systems to demonstrate the presence of a 38.5-kDa

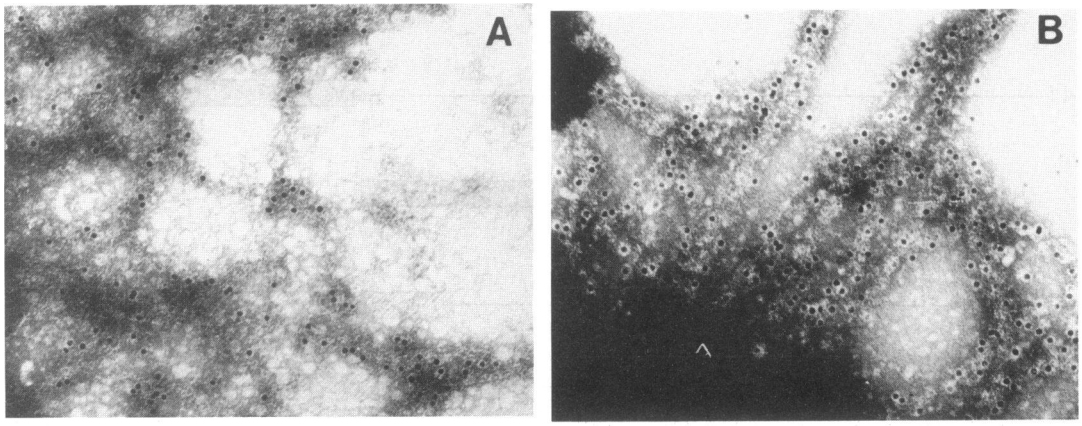


FIG. 3. Electron micrographs after colloidal gold immunolabeling of *E. coli* HB101(pPLHD52) (A) and *E. coli*(pPLHD53) (B). Bar, 0.1 μm.

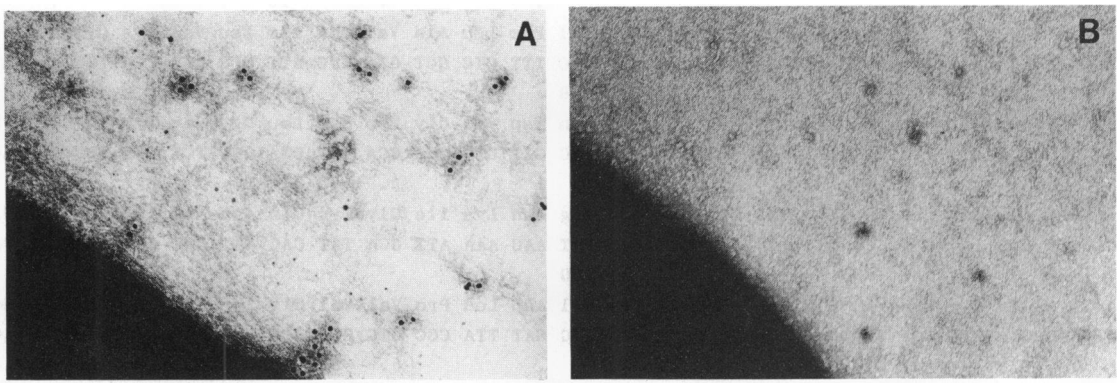


FIG. 4. (A) Immunogold electron microscopy studies of strain K514(pPLHD52) with adsorbed F17 antibodies (F17-G fraction). (B) Immunogold studies of strain K514(pPLHD53) with identical antibodies.

protein. The resulting autoradiographs are shown in Fig. 6. Minicell analysis of pPLHD2 and pPLHD50 clearly showed the presence of this peptide as a double band, suggesting the presence of a signal sequence (14) (Fig. 6A). Protein-labeling experiments of pPLHD53 demonstrated the loss of the 38.5 kDa protein (Fig. 6B). The 38.5-kDa protein could not be visualized on an overloaded SDS-polyacrylamide gel stained with Coomassie blue or silver when preparations consisting of purified fimbriae obtained from K514(pPLHD2) were analyzed.

In *trans* complementation of pPLHD55 with F17-G gene. The F17-G gene was subcloned by digesting pPLHD52 DNA with *Nsi*I. The 1.2-kb *Nsi*I fragment containing the F17-G gene was eluted from agarose gel and subcloned in the *Sma*I site of pUC9. Double digestions with *Hin*dIII-*Hpa*I of several transformants demonstrated the correct orientation behind the LacZ promoter of the *Nsi*I insert DNA in one of the clones. This construction was designated pPLHD101 (Fig. 1). In vitro transcription-translation analysis of pPLHD101 showed the presence of the 38.5-kDa protein (Fig. 6C).

The intact F17 gene cluster of pPLHD52 and the F17 gene cluster mutated in F17-G were inserted in pACYC184 (pPLHD54 and pPLHD55, respectively). In *trans* complementation of pPLHD55 with pPLHD101 resulted in piliated bacteria characterized by a NAG-dependent adherence to calf intestinal villi and coated Eupergit-C spheres similar to the adherence of HB101(pPLHD54) (Table 1).

Comparison of F17-G adhesin with MrkD (8), FimH (16), PapG (23), and F17-A (21) proteins. The amino acid sequences of MrkD, FimH, PapG, and F17-A are shown in Fig. 7. When F17-G was compared with PapG and FimH, 21% of the amino acids could be aligned; in the alignment of F17-G with MrkD, 17% of the residues were in similar positions. It is interesting that alignment of the F17-A subunit protein with the C-terminal residues of the F17-G adhesin protein resulted in 24% aligned amino acids. The 25 C-terminal residues of the F17-G, F17-A, PapG, FimF, and MrkD proteins demonstrated a high degree of similarity.

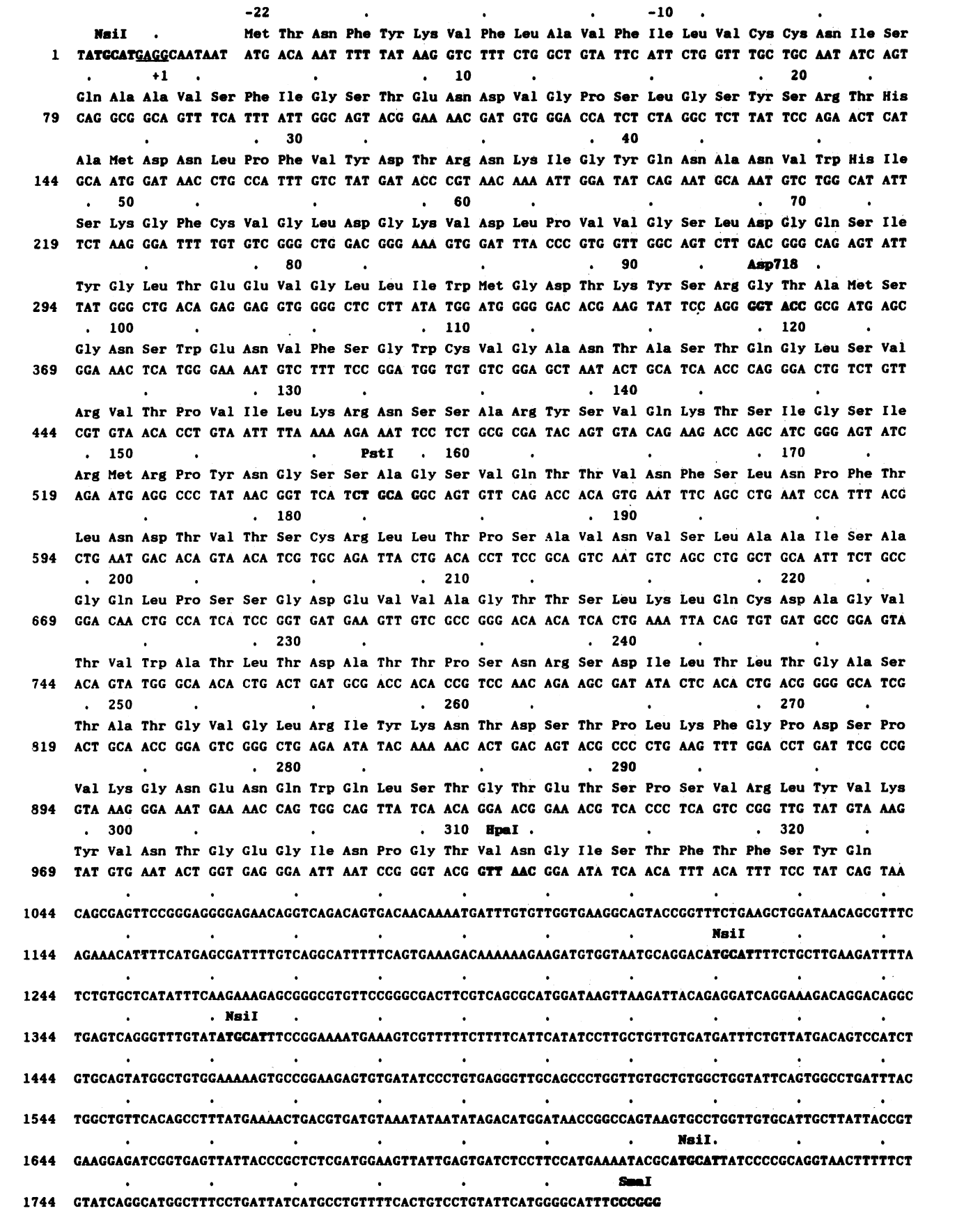
DISCUSSION

It has been generally accepted that the fimbrial subunit not only is essential for fimbrial structure but also carries the information for receptor binding. Recently, it was shown that certain mutations in cistrons on a chromosomal DNA fragment encoding Pap pili affected adhesion but did not abolish Pap pilus formation. Analogous findings were reported for type 1 and S fimbriae from *E. coli* and MrkD from *Klebsiella* sp. (8, 13, 16, 19, 22–24, 26, 27, 29, 32, 33, 39).

The present report describes genetic evidence illustrating that the F17 fimbriae found on the surface of bovine enterotoxigenic *E. coli* strains consist at least of the F17-A subunit protein and a distinct entity acting as the receptor-recognizing adhesin. Neither pPLHD50 nor pPLHD51, which carried a deletion in the F17-A subunit gene, could direct synthesis of functional fimbriae. In contrast, pPLHD53, mutated in the F17-G gene, directed the synthesis of morphologically intact fimbriae which could not be distinguished by electron microscopy from the fimbriae produced by HB101(pPLHD52). However, HB101(pPLHD53) failed to adhere to isolated calf intestinal villi and ovomucoid-coated Eupergit-C spheres. Polyclonal antibodies directed against F17-A protein stained both HB101(pPLHD52) and HB101(pPLHD53) in an identical manner. Furthermore, when equal numbers of HB101 (pPLHD52) and HB101(pPLHD53) were analyzed in a standard ELISA with polyclonal or monoclonal F17 antibodies, equal amounts of fimbrial protein were detected (Table 1). Polyacrylamide gel electrophoretic analysis could not distinguish between purified fimbriae of HB101(pPLHD52) and HB101(pPLHD53). These findings indicate that fimbriae on the adhesive and nonadhesive clones are very similar. Furthermore, we did gene knockout experiments with the F17-D and F17-C genes separately (19a). Mutation of the F17-C or the F17-D gene resulted in nonpiliated bacteria, indicating that, as is the case with the F17-A mutants, the F17-G adhesin cannot reach the cell surface or be exposed at this surface in the absence of the F17-C or F17-D protein. These experiments indicated that normal F17 piliation is mediated by the F17-A, F17-C, and F17-D genes, while the F17-G gene encodes for binding properties of the F17 pilus.

Complementation experiments were done to determine whether the lesion in pPLHD53 could be complemented in *trans*. The pACYC184 derivative plasmid carrying the mutated F17-G gene (pPLHD55) was cotransformed with pPLHD101 carrying the isolated F17-G gene positioned behind the *lacZ* promoter of pUC8. Colonies carrying both constructs gave rise to normal adhesive F17 fimbriae. Therefore, we were able to determine that the F17-G gene product is solely responsible for the NAG-sensitive receptor-binding phenotype.

Since we demonstrated that purified F17 fimbriae obtained from *E. coli* (pPLHD2) block the receptor on isolated calf villi (20), the adhesin component will be present on these


-22
-10
NsiI
Met Thr Asn Phe Tyr Lys Val Phe Leu Ala Val Phe Ile Leu Val Cys Cys Asn Ile Ser
1 TATGCATGAGGCAATAAT ATG ACA AAT TTT TAT AAG GTC TTT CTG GCT GTA TTC ATT CTG GTT TGC TGC AAT ATC AGT
+1
10
20
Gln Ala Ala Val Ser Phe Ile Gly Ser Thr Glu Asn Asp Val Gly Pro Ser Leu Gly Ser Tyr Ser Arg Thr His
79 CAG GCG GCA GTT TCA TTT ATT GGC AGT ACG GAA AAC GAT GTG GGA CCA TCT CTA GGC TCT TAT TCC AGA ACT CAT
30
40
Ala Met Asp Asn Leu Pro Phe Val Tyr Asp Thr Arg Asn Lys Ile Gly Tyr Gln Asn Ala Asn Val Trp His Ile
144 GCA ATG GAT AAC CTG CCA TTT GTC TAT GAT ACC CGT AAC AAA ATT GGA TAT CAG AAT GCA AAT GTC TGG CAT ATT
50
60
70
Ser Lys Gly Phe Cys Val Gly Leu Asp Gly Lys Val Asp Leu Pro Val Val Gly Ser Leu Asp Gly Gln Ser Ile
219 TCT AAG GGA TTT TGT GTC GGG CTG GAC GGG AAA GTG GAT TTA CCC GTG GTT GGC AGT CTT GAC GGG CAG AGT ATT
80
90
Asp718
Tyr Gly Leu Thr Glu Glu Val Gly Leu Leu Ile Trp Met Gly Asp Thr Lys Tyr Ser Arg Gly Thr Ala Met Ser
294 TAT GGG CTG ACA GAG GAG GTG GGG CTC CTT ATA TGG ATG GGG GAC ACG AAG TAT TCC AGG GGT ACC GCG ATG AGC
100
110
120
Gly Asn Ser Trp Glu Asn Val Phe Ser Gly Trp Cys Val Gly Ala Asn Thr Ala Ser Thr Gln Gly Leu Ser Val
369 GGA AAC TCA TGG GAA AAT GTC TTT TCC GGA TGG TGT GTC GGA GCT AAT ACT GCA TCA ACC CAG GGA CTG TCT GTT
130
140
Arg Val Thr Pro Val Ile Leu Lys Arg Asn Ser Ser Ala Arg Tyr Ser Val Gln Lys Thr Ser Ile Gly Ser Ile
444 CGT GTA ACA CCT GTA ATT TTA AAA AGA AAT TCC TCT GCG CGA TAC AGT GTA CAG AAG ACC AGC ATC GGG AGT ATC
150
PstI
160
170
Arg Met Arg Pro Tyr Asn Gly Ser Ser Ala Gly Ser Val Gln Thr Thr Val Asn Phe Ser Leu Asn Pro Phe Thr
519 AGA ATG AGG CCC TAT AAC GGT TCA TCT GCA GGC AGT GTT CAG ACC ACA GTG AAT TTC AGC CTG AAT CCA TTT ACG
180
190
Leu Asn Asp Thr Val Thr Ser Cys Arg Leu Leu Thr Pro Ser Ala Val Asn Val Ser Leu Ala Ala Ile Ser Ala
594 CTG AAT GAC ACA GTA ACA TCG TGC AGA TTA CTG ACA CCT TCC GCA GTC AAT GTC AGC CTG GCT GCA ATT TCT GCC
200
210
220
Gly Gln Leu Pro Ser Ser Gly Asp Glu Val Val Ala Gly Thr Thr Ser Leu Lys Leu Gln Cys Asp Ala Gly Val
669 GGA CAA CTG CCA TCA TCC GGT GAT GAA GTT GTC GCC GGG ACA ACA TCA CTG AAA TTA CAG TGT GAT GCC GGA GTA
230
240
Thr Val Trp Ala Thr Leu Thr Asp Ala Thr Thr Pro Ser Asn Arg Ser Asp Ile Leu Thr Leu Thr Gly Ala Ser
744 ACA GTA TGG GCA ACA CTG ACT GAT GCG ACC ACA CCG TCC AAC AGA AGC GAT ATA CTC ACA CTG ACG GGG GCA TCG
250
260
270
Thr Ala Thr Gly Val Gly Leu Arg Ile Tyr Lys Asn Thr Asp Ser Thr Pro Leu Lys Phe Gly Pro Asp Ser Pro
819 ACT GCA ACC GGA GTC GGG CTG AGA ATA TAC AAA AAC ACT GAC AGT ACG CCC CTG AAG TTT GGA CCT GAT TCG CCG
280
290
Val Lys Gly Asn Glu Asn Gln Trp Gln Leu Ser Thr Gly Thr Glu Thr Ser Pro Ser Val Arg Leu Tyr Val Lys
894 GTA AAG GGA AAT GAA AAC CAG TGG CAG TTA TCA ACA GGA ACG GAA ACG TCA CCC TCA GTC CGG TTG TAT GTA AAG
300
310
HpaI
320
Tyr Val Asn Thr Gly Glu Gly Ile Asn Pro Gly Thr Val Asn Gly Ile Ser Thr Phe Thr Phe Ser Tyr Gln
969 TAT GTG AAT ACT GGT GAG GGA ATT AAT CCG GGT ACG GTT AAC GGA ATA TCA ACA TTT ACA TTT TCC TAT CAG TAA
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NsiI
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1244 TCTGTGCTCATATTTCAAGAAAGAGCGGGCGTGTTCCGGGCGACTTCGTCAGCGCATGGATAAGTTAAGATTACAGAGGATCAGGAAAGACAGGACAGGC
NsiI
1344 TGAGTCAGGGTTTGTATATGCATTTCCGGAAAATGAAAGTCGTTTTTCTTTTCATTCATATCCTTGCTGTTGTGATGATTTCTGTTATGACAGTCCATCT
1444 GTGCAGTATGGCTGTGGAAAAAGTGCCGGAAGAGTGTGATATCCCTGTGAGGGTTGCAGCCCTGGTTGTGCTGTGGCTGGTATTCAGTGGCCTGATTTAC
1544 TGGCTGTTCACAGCCTTTATGAAAACTGACGTGATGTAAATATAATATAGACATGGATAACCGGCCAGTAAGTGCCTGGTTGTGCATTGCTTATTACCGT
NsiI
1644 GAAGGAGATCGGTGAGTTATTACCCGCTCTCGATGGAAGTTATTGAGTGATCTCCTTCCATGAAAATACGCATGCATTATCCCCGCAGGTAACTTTTTCT
SmaI
1744 GTATCAGGCATGGCTTTCCTGATTATCATGCCTGTTTTCACTGTCCTGTATTCATGGGGCATTTCCCGGG

FIG. 5. Nucleotide sequence of the F17-G gene. The deduced amino acid sequence is indicated above the nucleotide sequence. Numbers above each line refer to the amino acid position. The first amino acid of the mature protein is numbered +1, and the amino acids of the putative signal sequences are indicated by negative numbers. A putative Shine-Dalgarno consensus sequence is underlined.

fimbriae. This was further demonstrated by the immunogold studies using anti F17-G antibodies which reacted preferentially with fimbriae encoded by the pPLHD52 plasmid and to a much less extent with fimbriae encoded by the F17-G mutant plasmid pPLHD53. These immunogold studies could not locate the F17-G protein at the tip of the pilus since F17 fimbriae are wiry.

The presence of a signal sequence on the F17-G peptide, documented by minicell analysis and sequence data, further supports the hypothesis that F17-G is a secreted protein normally located within the fimbrial structure. The F17-G protein is a minor fimbrial component acting as an adhesin protein or as an F17-A-modifying protein. These modifications, when they occur, are probably minor since no morphological changes could be observed between the intact functional fimbriae and the nonadhesive fimbriae.

Our results further confirm reports indicating important amino acid sequence homology between fimbrial adhesins (8). Important similarities of these adhesins are the presence of four cysteines in the protein suggesting a two-domain structure, a conserved glycine 14 amino acids from the C terminus, and a conserved aromatic amino acid at the penultimate position. Furthermore, significant sequence homology was present between the F17-G adhesin and the F17-A major subunit pilin.

Our results indicated also that an F17 pilus structure is required for binding in addition to the F17-G protein. Minicell analysis of pPLHD52 demonstrated that the absence of the major subunit peptide does not affect the expression of other proteins of the F17 genome. It is also possible that the major pilus subunit protein is indispensable for the F17-G adhesion to reach the bacterial cell surface.

As a general conclusion, F17-mediated binding requires the presence of an intact pilus structure together with the binding moiety. Since the F17 fimbriae (F17-A) are characterized by high hydrophobicity and bind to calf mucus (albeit much more weakly than K99 fimbriae) (31), we speculate that the F17-mediated binding to the calf intestine occurs by hydrophobic interactions between pilus subunit and mucus followed by NAG-dependent adhesion to the epithelial carbohydrate receptors. This specific adhesion is mediated by the F17-G gene product.

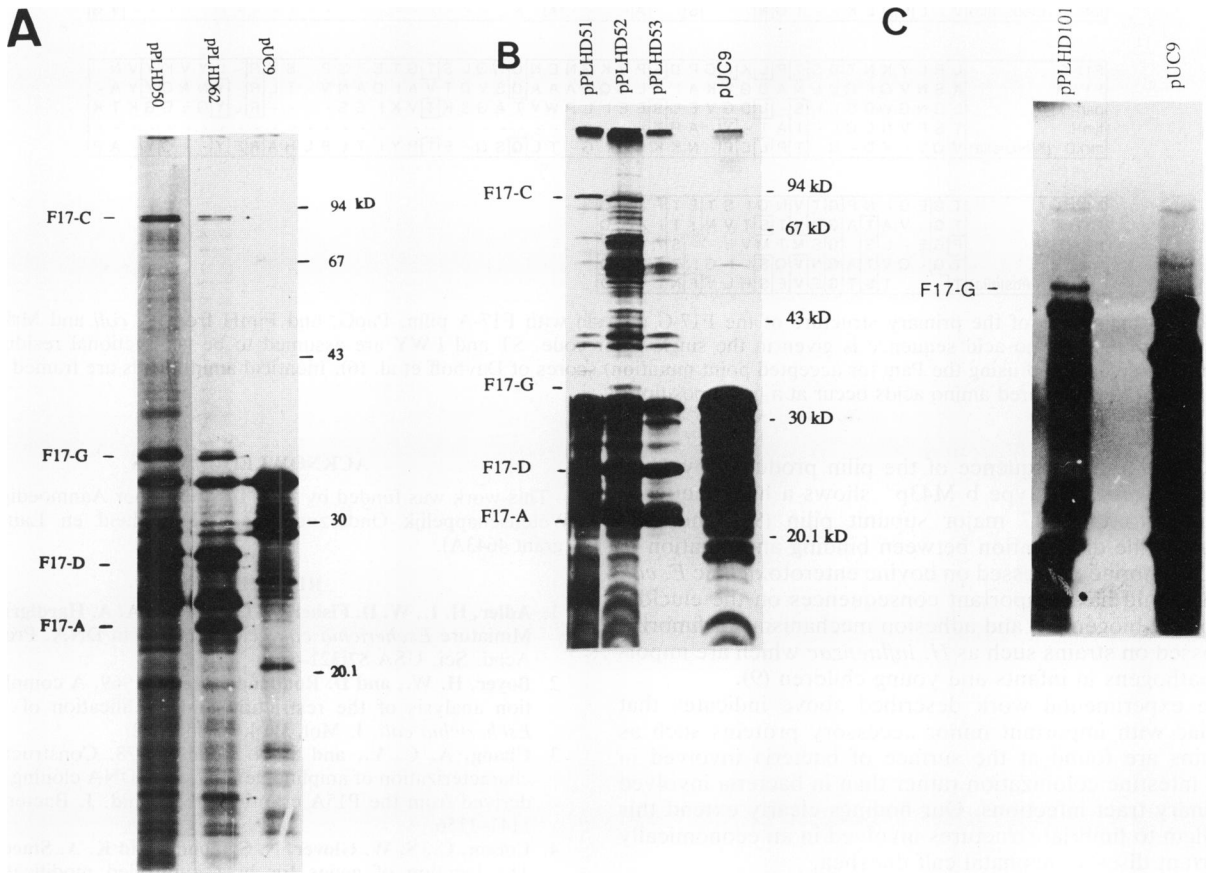

FIG. 6. (A) Minicell analysis of proteins expressed from pUC9, pPLHD52, and pPLHD50. (B) Autoradiograph of labeled proteins expressed in a cell-free transcription-translation system. The gene products from pUC9, pPLHD52, and pPLHD53 are shown. (C) Autoradiograph is overexposed to demonstrate the weakly expressed F17-G protein encoded by pPLHD101. The pUC9 products are shown as a control; molecular sizes of standard proteins are marked in kilodaltons (kDa). A, F17-A subunit; G, F17-G adhesin.

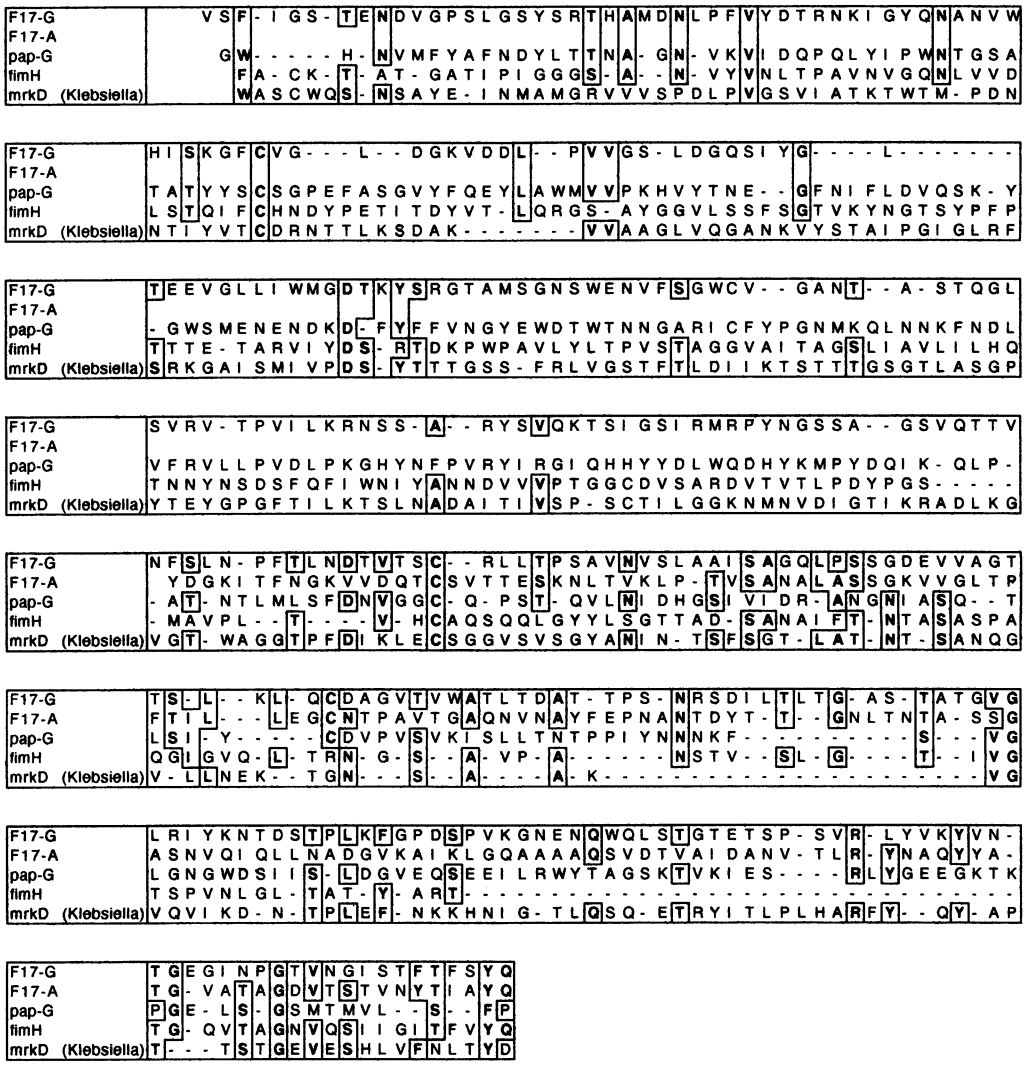

FIG. 7. Comparison of the primary structure of the F17-G adhesin with F17-A pilin, PapG, and FimH from *E. coli* and MrkD from *Klebsiella* sp. The amino acid sequence is given in the single-letter code. ST and FWY are assumed to be isofunctional residues. The sequences are compared using the Pam (or accepted point mutation) scores of Dayhoff et al. (6). Identical amino acids are framed when at least three of the compared amino acids occur at a given position.

The amino acid sequence of the pilin produced by *Haemophilus influenzae* type b M43p$^+$ shows a high sequence similarity to the F17 major subunit pilin (9). The data indicating the dissociation between binding and piliation in the F17 fimbriae expressed on bovine enterotoxigenic *E. coli* strains could have important consequences on the elucidation of the biogenesis and adhesion mechanisms of fimbriae expressed on strains such as *H. influenzae* which are important pathogens in infants and young children (9).

The experimental work described above indicates that fimbriae with important minor accessory proteins such as adhesins are found at the surface of bacteria involved in small intestine colonization rather than in bacteria involved in urinary tract infections. Our findings clearly extend this paradigm to fimbrial structures involved in an economically important disease, neonatal calf diarrhea.

It is our intention to perform protection studies using the F17-G protein or anti-F17-G Fab fragments in order to evaluate the role of this fimbrial component in a new generation of vaccines.

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REFERENCES

1. **Adler, H. I., W. D. Fisher, A. Cohen, and A. A. Hardigree.** 1967. Miniature *Escherichia coli* cells deficient in DNA. Proc. Natl. Acad. Sci. USA **57**:321–326.
2. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41**:459–472.
3. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**: 1141–1156.
4. **Colson, C., S. W. Glover, N. Symonds, and K. A. Stacey.** 1965. The location of genes for host controlled modification and restriction in *E. coli* K12. Genetics **52**:1043–1050.
5. **Contrepois, M. G., and J. P. Girardeau.** 1985. Additive protective effects of colostral antipili antibodies in calves experimentally infected with enterotoxigenic *Escherichia coli*. Infect.

Immun. 50:947–949.
6. **Dayhoff, M. O., R. M. Schwartz, and B. C. Orcutt.** 1978. *In* M. O. Dayhoff (ed.), Atlas of protein sequence and structure, vol. 5, suppl. 3, p. 345–352. National Biomedical Research Foundation, Washington, D.C.
7. **Gaastra, W., and F. K. De Graaf.** 1982. Host-specific fimbrial adhesins of noninvasive enterotoxigenic *Escherichia coli* strains. Microbiol. Rev. **46**:129–161.
8. **Gerlach, G.-F., S. Clegg, and B. L. Allen.** 1989. Identification and characterization of the genes encoding the type 3 and type 1 fimbrial adhesins of *Klebsiella pneumoniae*. J. Bacteriol. **171**:1262–1270.
9. **Gilsdorf, J. R., C. F. Marrs, K. W. McCrea, and L. J. Forney.** 1990. Cloning, expression, and sequence analysis of the *Haemophilus influenzae* type b strain M43p$^+$ pilin gene. Infect. Immun. **58**:1065–1072.
10. **Girardeau, J. P.** 1980. A new in vitro technique for attachment to intestinal villi using enteropathogenic *E. coli*. Ann. Microbiol. (Inst. Pasteur) **131B**:31–37.
11. **Girardeau, J. P., H. C. Dubourguier, and M. Contrepois.** 1979. Attachement des *E. coli* entéropathogènes à la muqueuse intestinale. Gastro-entérites Néonatales Veau **25–26 October**:53–66.
12. **Gronenborn, B., and J. Messing.** 1978. Methylation of single-stranded DNA in vitro introduces new restriction endonuclease cleavage sites. Nature (London) **272**:375–377.
13. **Hanson, M. S., and C. Brinton, Jr.** 1988. Identification and characterization of *E. coli* type-1 pilus tip adhesion protein. Nature (London) **332**:265–268.
14. **Inouye, M., and S. Halegoua.** 1980. Secretion and membrane localization of proteins in *Escherichia coli*. Crit. Rev. Biochem. **7**:339–371.
15. **Klemm, P.** 1985. Fimbrial adhesins of *E. coli*. Rev. Infect. Dis. **7**:321–340.
16. **Klemm, P., and G. Christiansen.** 1987. Three fim genes required for the regulation of length and mediation of adhesion of *E. coli* type 1 fimbriae. Mol. Gen. Genet. **208**:439–445.
17. **Korhonen, T. K., E.-L. Nurmiaho, H. Ranta, and C. Svanborg Edén.** 1980. New method for isolation of immunological pure fimbriae from *Escherichia coli*. Infect. Immun. **27**:569–575.
18. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
19. **Lindberg, F., B. Lund, L. Johansson, and S. Normark.** 1987. Localization of the receptor-binding protein adhesin at the tip of the bacterial pilus. Nature (London) **328**:84–87.
19a. **Lintermans, P. F., et al.** Unpublished data.
20. **Lintermans, P. F., P. Pohl, A. Bertels, G. Charlier, J. Vande Kerckhove, J. Vandamme, J. Schoup, C. Schlicker, T. Korhonen, H. De Greve, and M. Van Montagu.** 1988. Characterization and purification of the F17 adhesin present on the surface of bovine enteropathogenic and septicemic *Escherichia coli*. Am. J. Vet. Res. **49**:1794–1799.
21. **Lintermans, P. F., P. Pohl, F. Deboeck, A. Bertels, C. Schlicker, J. Vande Kerckhove, J. Vandamme, M. Van Montagu, and H. De Greve.** 1988. The isolation and nucleotide sequence of the F17-A gene encoding the structural protein of the F17 fimbriae in bovine enterotoxigenic *Escherichia coli*. Infect. Immun. **56**: 1475–1484.
22. **Lund, B., F. Lindberg, M. Båga, and S. Normark.** 1985. Globoside-specific adhesins of uropathogenic *Escherichia coli* are encoded by similar *trans*-complementable gene clusters. J. Bacteriol. **162**:1293–1301.
23. **Lund, B., F. Lindberg, B.-I. Marklund, and S. Normark.** 1987. The PapG protein is the α-D-galactopyranosyl-(1→4)-β-D-galactopyranose-binding adhesin of uropathogenic *E. coli*. Proc. Natl. Acad. Sci. USA **84**:5898–5902.
24. **Lund, B., F. Lindberg, and S. Normark.** 1988. Structure and antigenic properties of the tip-located P pilus proteins of uropathogenic *Escherichia coli*. J. Bacteriol. **170**:1887–1894.
25. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Maurer, L., and P. Orndorff.** 1985. A new locus, pilE, required for the binding of type 1 piliated *Escherichia coli* to erythrocytes. FEMS Microbiol. Lett. **30**:59–66.
27. **Maurer, L., and P. Orndorff.** 1987. Identification and characterization of genes determining receptor binding and pilus length of *Escherichia coli* type 1 pili. J. Bacteriol. **169**:640–645.
28. **Maxam, A. M., and W. Gilbert.** 1977. A new method for sequencing DNA. Proc. Natl. Acad. Sci. USA **74**:560–564.
29. **Minion, F. C., S. N. Abraham, E. H. Beachey, and J. D. Goguen.** 1986. The genetic determinant of adhesive function in type 1 fimbriae of *Escherichia coli* is distinct from the gene encoding the fimbrial subunit. J. Bacteriol. **165**:1033–1036.
30. **Mooi, F. R., and F. K. De Graaf.** 1985. Molecular biology of fimbriae of enterotoxigenic *Escherichia coli*. Curr. Top. Microbiol. Immunol. **118**:119–135.
31. **Mouricout, M. A., and R. A. Julien.** 1987. Pilus-mediated binding of bovine enterotoxigenic *E. coli* to calf small intestinal mucins. Infect. Immun. **55**:1216–1223.
32. **Norgren, M., S. Normark, D. Lark, P. O'Hanley, G. Schoolnik, S. Falkow, C. Svanborg-Edén, M. Båga, and B. E. Uhlin.** 1984. Mutations in *E. coli* cistrons affecting adhesion to human cells do not abolish Pap pili fiber formation. EMBO J. **3**:1159–1165.
33. **Normark, S., M. Båga, M. Goransson, F. P. Lindberg, B. Lund, M. Norgren, and B.-E. Uhlin.** 1986. Genetics and biogenesis of *Escherichia coli* adhesins p. 113–143. *In* D. Mirelman (ed.), Microbial lectins and agglutinins. Wiley Interscience, New York.
34. **Orndorff, P. E., P. A. Spears, D. Schauer, and S. Falkow.** 1985. Two modes of control of *pilA*, the gene encoding type 1 pilin in *Escherichia coli*. J. Bacteriol. **164**:321–330.
35. **Pohl, P., P. Lintermans, K. Van Muylem, and M. Schotte.** 1982. Colibacilles entérotoxigènes de veau possèdant un antigène d'attachement différent de l'antig**126**:569–571.
36. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
37. **Swinghamer, E. A.** 1980. A method for improved lysis of some gram-negative bacteria. FEMS Microbiol. Lett. **7**:157–162.
38. **Thompson, R., and M. Achtman.** 1978. The control region of the F sex factor DNA transfer cistrons: restriction mapping and DNA cloning. Mol. Gen. Genet. **165**:295–304.
39. **Uhlin, B. E., M. Norgren, M. Båga, and S. Normark.** 1985. Adhesion to human cells by *Escherichia coli* lacking the major subunit of a digalactoside-specific pilus-adhesin. Proc. Natl. Acad. Sci. USA **82**:1800–1804.
40. **Van Driessche, E., J. Schoup, G. Charlier, P. Lintermans, S. Beeckmans, R. Zeeuws, P. Pohl, and L. Kanarek.** 1988. The attachment of *E. coli* F17$^+$ to intestinal calf villi and to eupergit-glycoprotein beads p. 55–61. *In* T. C. Bog-Hansen and D. L. J. Freed (ed.), Lectins—biology, biochemistry, clinical biochemistry, vol. 6. Sigma Press, St. Louis, Mo.
41. **Vieira, J., and J. Messing.** 1982. The pUC plasmids; an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primer. Gene **19**:259–268.